Amendents to the speficiation

Please amend the specification as follows:

Please amend the paragraph at column 2, starting at line 5 as follows:

α-hordothionin is a 45-amino acid protein which has been well characterized. It can be isolated from seeds of barley (*Hordeum vulgare*) and even in its native form is especially rich in arginine and lysine residues, containing 5 residues (10%) of each. The amino acid sequence is as provided in SEQUENCE I.D. No. 1. It has powerful antifungal properties. Initial work to enhance the lysine content of this protein provided a high lysine derivative as indicated in SEQUENCE I.D. No. 2. However, it was impossible to predict the ultimate effect of this seemingly trivial substitution on the tertiary structure and folding of the protein, and subsequent bioassays determined that this derivative did not fold to a biologically active species in vitro. In addition, both tertiary structure and folding are critical to the stability and adequate expression of the protein in vivo, and both were absent in this compound. Therefore, further analysis and functional modeling of the wild-type compound was undertaken to determine whether substitutions could be made without disrupting biological activity. Although the crystal structure of crambin, a small protein of similar size and structure, has been reported, such crystal structures have not previously been available for hordothionin or even related compounds such as purothionin and viscotoxin. We undertook to develop such structural information.

Please amend the paragraph at column 2, starting at line 28, as follows:

Three-dimensional modeling of the protein led us to believe that the arginine residue at position 10 was critical to retention of the appropriate 3-dimensional structure and possible folding through hydrogen bond interactions with the C-terminal residue of the protein. A lysine substitution at that point with its shorter side chains could not hydrogen bond at the same time to both the serine residue at the 2 position and to the C-terminus while maintaining the backbone structure which we had predicted. The synthetic peptide having this substitution could not be made to fold correctly, which supported this analysis. Conservation of the arginine residue at position 10 provided a protein which folded correctly, had the sequence indicated in SEQUENCE I.D. No. 3, and exhibited antifungal

activity in a bioassay. Comparison of the structure of hordothionin with that of the loosely related (48% homologous, 30% identical) protein crambin showed that thionin had a disulfide bond linking the cysteines at positions 12 and 29 which was not bridging the corresponding positions in crambin. Accordingly, replacement of the cysteine at position 12 of thionin with lysine and replacement of the cysteine at position 29 with threonine to produce a protein having the sequence indicated in SEQUENCE I.D. No. 4 was found not to disrupt the 3-dimensional structure of the protein, as evidenced by an energy content which was determined to be indistinguishable from that of the native protein ~~however, substitution at position 12 did not work *in vivo*~~.

Please amend the paragraph at column 2, starting at line 53 as follows:

Further analysis of substitutions which would not alter the 3-dimensional structure of the molecule led to replacement of Asparagine-11, Glutamine-22 and Threonine-41 with lysine residues with virtually no steric hindrance. The resulting compound had the sequence indicated in SEQUENCE I.D. No. 5, containing 29% lysine residues. In addition, it was 1determined that by replacement of the serine residue at position 2 with aspartic acid, the arginine at position 10 could be replaced with lysine while permitting the needed hydrogen bonding with the C-terminus, providing a compound of the sequence indicated in SEQUENCE I.D. No. 6. It should be appreciated that these substitutions would be effective and acceptable and could not have been predicted by examination of the linear sequence of the native thionin protein ~~however, substitution at position 10 did not work *in vivo*~~.

Please amend the paragraph at column 3, starting at line 1 as follows:

Other combinations of these substitutions were also made, providing proteins having the sequences indicated in SEQUENCE ID NO: 7 and SEQUENCE ID NO. 8. Accordingly, this invention provides proteins having the sequence of. SEQUENCE ID NO: 1 wherein the amino acid residues at one or more of positions 5, 11, 17, 19, 22, 30 and 41 are lysine, and the remainder of the residues at those positions are the residues at the corresponding positions in SEQUENCE ID NO: 1, provided that the residue at position 30 is threonine when the residue at position 12 is lysine and cysteine otherwise, and the residue at position 2 is aspartic acid when the residue at position 10 is lysine and serine otherwise. Although the native

hordothionin is relatively lysine rich, a storage protein with 10% lysine residues (by number) cannot be expressed at high enough levels to obtain total protein lysine contents which are sufficient to obviate the need for lysine supplementation in poultry and swine feeds. These compounds are significantly more lysine enriched, and can be made to contain nearly thirty percent lysine residues. Without such enhanced lysine contents, it is impossible to eliminate the need for lysine supplementation of feeds. This invention thus also provides an important method for enhancing the lysine content of a plant cell or a plant, comprising the step of causing one or more proteins according to this invention to be expressed in the cell or plant.